SOLUBILIZED CONTACT SITES A FROM CELL MEMBRANES OF *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

Cell-to-cell adhesion in aggregating cells of the slime mold *Dictyostelium discoideum* can be completely inhibited by the combined action of univalent antibody fragments (F_{ab}) of two different specificities. The binding sites of these antibodies at the cell surface have been referred to as contact sites A and B, respectively [1]. In contrast to contact sites B which are present both in the growth phase stage and in aggregating cells, the A sites are developmentally regulated. They become detectable when the cells differentiate into the aggregation-competent stage. F_{ab}-fragments against contact sites A specifically block the EDTA-resistant type of cell adhesion which is characteristic of aggregating cells. In the present paper we report the solubilization and partial purification of contact sites A.

Similar to contact sites A, cyclic-AMP phosphodiesterase at the cell surface is strongly regulated during cell differentiation [2,3]. This enzyme functions in cutting off cyclic-AMP pulses which are periodically generated and transmitted between aggregating cells [4]. Our results indicate that the cell-surface phosphodiesterase activity does not reside in the contact sites A.

2. Methods

2.1. *Cell culture*

Cells of the axenic strain Ax-2 were cultivated in nutrient medium and washed in phosphate buffer for the induction of cell differentiation [5]. Inhibition of cell aggregation by trypsin (Worthington, 1 × cryst.) was tested using 10^7 cells per ml either in barbital buffer pH 7.3 plus 10 mM EDTA [1] or in 17 mM phosphate buffer pH 6.8 containing 10 mM EDTA plus 2% glycerol [6]. Trypsin concentrations were 0.1, 0.5 and 1 mg per ml, and aggregation inhibition was measured [1] over a period of 2 hr.

2.2. *Assays*

Cyclic-AMP phosphodiesterase activity was determined by a coupled enzyme assay [7], and expressed in units of nmoles cyclic AMP hydrolyzed per minute at 35°C [8]. Contact sites A were measured by absorption of aggregation-inhibition F_{ab}, followed by retitration of its aggregation-inhibiting activity using clone M 2 cells in the presence of 10 mM EDTA [1]. The quantity of contact sites A at the surface of 10^6 aggregation-competent Ax-2 cells was taken as 1 unit. Protein was determined using the Lowry method. Crude solutions of the carbohydrate-binding protein, Discoidin [17], were prepared by sonication of cells and centrifugation at 150 000 *g*, and assayed using formalinized sheep erythrocytes [9]. The Discoidin was pre-incubated with the contact site A fraction for 20 min at room temperature for testing of agglutination inhibition.

2.3. *Solubilization*

Cells were frozen 3 times and thawed in 0.017 M phosphate buffer pH 6.0, and either the 27 000 *g* sediment [1] or a 700 *g* sediment prepared according to [10] was used for solubilization. Solubilization procedures: (a) 10 ml suspension of 27 000 *g* sedi-

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ment in the phosphate buffer was shaken with 7.5 ml *n*-butanol at 4°C, and the clear water phase was recovered; or (b) either the 700 or 27 000 *g* sediment was resuspended in 0.05 M Tris–HCl buffer pH 7.8 containing either 1% Triton X-100 or 1% sodium desoxycholate and incubated for 1 hr at 4°C. In both cases the 100 000 *g* supernatant was used as a solubilized fraction. Before the assay of contact sites both detergents were removed by bio-beads SM 2 [11] followed by dialysis.

2.4. *Purification*

Before chromatography on Whatman DE cellulose 32, the desoxycholate was reduced to 0.75%, and the column eluted by a KCL gradient in 10 mM Tris-·HCl buffer pH 7.8 plus 0.1% desoxycholate. Sephadex G 200 columns were run in the same buffer plus 0.3 M KCl and 0.1% desoxycholate.

3. Results

3.1. *Solubilization of contact sites A*

By various treatments of living cells only small yields of contact sites A were obtained (table 1). The ineffectiveness of trypsinization in liberating serologically active contact sites was accompanied by its ineffectiveness in inhibiting cell-to-cell adhesion of aggregation competent cells.

Extraction of membrane fractions with *n*-butanol yielded solubilized contact sites A of the highest specific activity, compared to both Triton X-100 and desoxycholate. The *n*-butanol extracted sites, however, strongly aggregated and were therefore difficult to purify. The cells used for the assay of contact sites were extremely sensitive against traces of Triton X-100 even after addition of 30% albumin [12]. Therefore purification was started from desoxycholate solubilized membranes.

3.2. *Purification of contact sites A and cyclic-AMP phosphodiesterase*

By chromatography of desoxycholate-solubilized membranes on DEAE-cellulose and Sephadex G 200, contact sites A were purified 160 fold relative to the activities measured at the surface of intact cells (table 2). DEAE-chromatography resulted in the partial separation of contact sites A and cyclic-AMP phosphodiesterase. The major 280 nm-absorbing material was removed from both these fractions (fig.1). On Sephadex G-200 the contact-site fraction eluted in one symmetrical peak corresponding to mol. wt of

Table 1
Solubilization of contact sites A

Source	Treatment	Percent of activity solubilized	Percent of total protein solubilized	Specific activity (units per mg protein)
Living aggregation-competent cells	1 hr 0.3 M NaCl	0.6	0.2	100
	1 hr 0.2 M urea	6	n.d.[a]	–
	1 hr 17 mM phosphate pH 6.0	6	n.d.	–
	4 hr 1 mM EDTA in 1 mM phosphate pH 6.0	10	0.4	120
	1 hr Trypsin 1 mg/ml	6	n.d.	–
	Barbital buffer pH 7.3	6	n.d.	–
27 000 *g* sediment of cell homogenate	*n*-butanol	30	5	up to 600
	Triton X-100	60	55	70
	Na-desoxycholate	85	80	up to 90

[a]n.d. – Not determined.

NaCl and urea treatment was accomplished in 17 mM phosphate buffer pH 6.0, trypsin treatment in barbital buffer pH 7.3. The protein content of the particle suspensions used for *n*-butanol extraction was 1.5 mg/ml, for Triton and desoxycholate extraction 2 mg/ml.

Table 2
Purification steps of contact sites A and membrane-bound cyclic-AMP phosphodiesterase

Preparation	Specific activities (units per mg protein)		
	Contact sites A	Discoidin	Phosphodiesterase
Aggregation-competent cells in vivo	14		
27 000 *g* sediment	60–70		70[a]
Desoxycholate extract, 100 000 *g* supernatant	80–90	400	50–60
DEAE-cellulose: contact sites A peak phosphodiesterase peak	1200–1500	<4	120
	<5		700–900
Sephadex G-200: contact sites A peak phosphodiesterase peak	approx. 2200		<50
			approx. 2100

[a] Value taken from [3]. Discoidin units are reciprocal titers of sheep erythrocyte agglutination [9].

120–130 000. The phosphodiesterase showed a particle weight of obviously aggregated material in the region of 200 000 (fig.2).

The F_{ab} preparation used for assay of contact sites A contained antibody specificities against two or possibly more different immunodeterminants of these sites [13]. The peak fraction from Sephadex absorbed at least 90% of the contact-site A blocking activity of this F_{ab} preparation. This result indicates that the purified factor contained, in one single fraction, the total antigenic material of contact sites A. The purified contact sites A did not agglutinate formalinized sheep erythrocytes, the unfractionated desoxycholate solubilized membranes, however, did so (Table 2). This result shows that the agglutinating factor, a carbohydrate-binding protein called. Discoidin [9], was

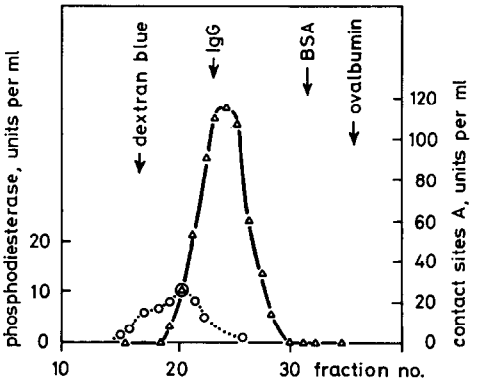

Fig.2. Elution profiles on Sephadex G 200. (△) contact sites A; (○) cyclic-AMP phosphodiesterase. 1.2 mg protein of the contact site A fraction from DE-cellulose was chromatographed on a 770 × 16 mm column.

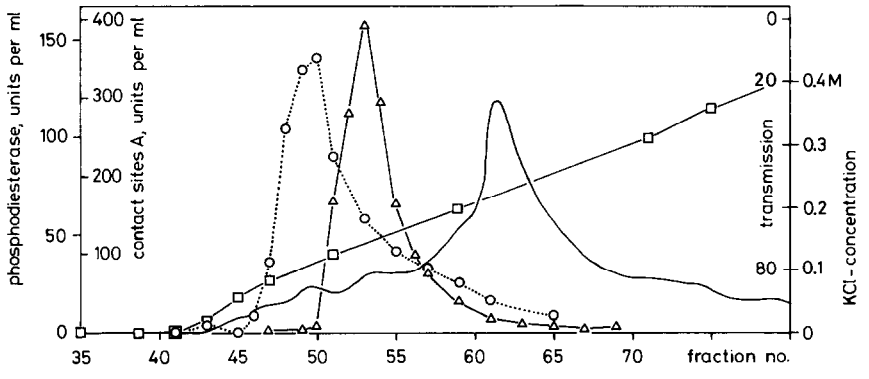

Fig.1. Chromatography of contact sites A and membrane-bound phosphodiesterase on DE-cellulose. (△) contact sites A; (○) cyclic-AMP phosphodiesterase; (□) KCl gradient. Solid line: percent transmission, scale from LKB unicord. 65 mg protein of desoxycholate solubilized 700 *g* sediment was added to a 350 × 12 column.

present in the crude solubilized fraction, but was no longer associated with the contact sites A after their purification. When pre-incubated with Discoidin, 900 units of purified contact sites A did not inhibit the agglutination of sheep erythrocytes by 1 or 2 units of Discoidin, indicating that the carbohydrate-binding site of Discoidin remained unblocked. (10^7 aggregation-competent cells contain approximately 200 units of Discoidin [9] and, at their surface, 10 units of contact sites A.

It has been suggested that Discoidin, a carbohydrate binding protein at the surface of aggregating *D. discoideum* cells, functions as a ligand between cells, thus establishing cell cohesiveness [17]. The mol. wt of Discoidin is 100 000 [9]. Contact sites A did not exhibit the properties of free discoidin, and in the state we have tested them did not function as discoidin receptors. Identity of contact sites A with the complex of Discoidin and its receptor remains a possibility to be tested.

4. Discussion

4.1. *Non-identity of contact sites A and cell-surface phosphodiesterase*

Evidence obtained in mutants indicated that there is a common step in the genetic control of both contact sites A and cellsurface phosphodiesterase [13]. One mechanism involved in this control is the periodic generation of cyclic-AMP pulses by differentiating cells [14]. Such pulses stimulate the appearance at the cell surface of both contact sites A and phosphodiesterase, which, however, do not always increase in identical temporal patterns [15]. The chromatographic separation of these two membrane sites definitely establishes that they are different molecular entities.

4.2. *Properties of contact sites A*

Cell-surface labelling with tritiated F_{ab} has indicated that contact sites A constitute a minor fraction of the cell-surface antigens in aggregating cells. These sites were completely blocked when not more than 2% of the cell surface was covered by F_{ab} molecules, corresponding to a number of 3×10^5 antibody sites per cell [16]. In agreement with these results contact sites A could be chromatographically separated from the major proteins present in desoxycholate-solubilized membranes.

Trypsin did not inhibit cell aggregation in suspensions of aggregation-competent cells. Re-aggregation of mechanically dissociated cells of a later developmental stage, the 'slug', is, however, inhibited by trypsin [6]. These results point at an unexplained between aggregation-competent cells and those obtained from dissociated slugs.

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